Aminotransferase Activity in Three Fresh Water Gastropods Subjected to Salinity Stress

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INTRODUCTION

In most of the invertebrates and vertebrates, the important pathway in the metabolism of amino acids seems to be the transaminase reaction, in which pairs of keto and amino acids are reciprocally aminated and deaminated. Furthermore, transaminase reactions form a link between carbohydrate and protein metabolism. Whereas extensive work has been done on transaminases in vertebrates, comparatively little has been carried out in invertebrates. Among mollusks, lamellibranchs form the major base for the study of aspartate and alanine aminotransferases (Hammen & Wilbur, 1959; Read, 1962, 1963; Awapara & Campbell, 1964; Hammen, 1968) with a few studies on gastropods (Zandee *et al.*, 1958; Awapara & Campbell, *op. cit.*). Since environmental salinity brings about changes in the concentration of free amino acids in mollusks (Allen, 1961; Lange, 1963; Lynch & Wood, 1966; Virkar & Webb, 1970) and other invertebrates (Florkin & Schoffeniels, 1965), it is of interest to study the enzymes associated with their metabolism in relation to imposed salinity stress.

MATERIALS AND METHODS

Preparation of the material: The gastropod snails *Lymnaea luteola, Planorbis* sp. and *Viviparus bengalensis* were collected from paddy fields around Tirupati and were brought to the laboratory in perforated wide-mouthed polythene bottles. They were isolated in about 25 ml of dechlorinated tap water in specimen tubes for 24 hours and periodically observed. The snails which shed cercariae were considered infected and others normal. Only normal snails were used for experimentation.

Salt water of 0.001, 0.002, 0.003, 0.004, and 0.005 M concentrations was prepared by proper dilution from a stock solution of sodium chloride. About 20 animals were placed in each concentration and acclimated for 10 days. Daily they were fed half-boiled leaves of *Amaranthus viridis*. After the 10th day, the animals were used for enzyme assay.

Enzyme assay: Aspartate aminotransferase (AAT : EC 2.6.1.1) and alanine aminotransferase (AlAT : EC 2.6. 1.2) activities were determined by the colorimetric method of Bergmeyer & Bernt, 1965. The reaction mixture contained in a total of 1 ml: 100 μmoles of phosphate buffer at pH 7.4, 100 μmoles of L-aspartate (substrate for AAT) or 50 μmoles of DL-alanine (substrate for AlAT), 2 μmoles of *L*-ketoglutarate and 0.2 ml of the tissue homogenate prepared in 0.25 M sucrose. The amounts of oxaloacetate or pyruvate formed from the substrates were measured on a Bausch & Lomb colorimeter at 545 μm. The enzyme activity is expressed in terms of μmoles of sodium pyruvate per mg protein per hr.

Protein determination: Protein content was determined by the Folin Phenol method of Lowry *et al.* (1951), using bovine albumin as standard. All the reagents used were of analytical grade supplied either by E. Merck A. G., Darmstadt, Germany, or by the British Drug House, England.

All the operations were performed at 4° C. The data were subjected to statistical analysis by calculating the Student's significance test. The probability levels above 0.05 were considered non-significant.

RESULTS

Salinity tolerance: *Lymnaea* were very susceptible to death even in 0.001 M salt water during summer. They survived well in all concentrations in winter. *Planorbis* and *Viviparus* exhibited tolerance to all saline media both in winter and in summer. No weight changes could be detected in any animal exposed to different saline media for 10 days.

Aspartate aminotransferase: The AAT activity decreased in the following order: *Lymnaea* < *Planorbis* < *Viviparus* (Table 1). On acclimation, *Lymnaea* showed decreased activity at lower concentrations and increased activity at higher concentrations, the increase being statistically non-significant. In *Planorbis* the enzyme activity was elevated at 0.001 M, followed by gradual decrease with increased salinity, the variations being non-significant. A similar trend was observed in *Viviparus*. However, in 0.005 M salinity the variation was significant.

Alanine aminotransferase: The activity of the enzyme is highest in *Lymnaea* and lowest in *Planorbis* (Table 2). The activity in *Lymnaea* decreased in lower saline media and increased in higher concentrations, the variation being significant at all concentrations except at 0.001 M salt water. *Planorbis* showed increased AlAT activity in 0.002 and 0.003 M saline media and had decreased activity at the lowest and highest concentrations, the variation being non-significant, except at 0.005 M salinity. But in *Viviparus* the activity was significantly elevated in all concentrations of salinity.

Table 1

Aspartate aminotransferase activity in tissue homogenates of snails in different concentrations of salinity

Animals	Control	Salinity Concentrations				
		0.001 M	0.002 M	0.003 M	0.004 M	0.005 M
Lymnaea	2.77 ± 0.32	1.47 ± 0.15	1.10 ± 0.11	2.86 ± 0.30	3.10 ± 0.47	3.00 ± 0.48
		P < 0.001	P < 0.001	NS	NS	NS
Planorbis	2.36 ± 0.29	3.00 ± 0.66	2.69 ± 0.46	2.71 ± 0.49	2.00 ± 0.43	2.02 ± 0.38
		NS	NS	NS	NS	NS
Viviparus	1.01 ± 0.18	1.20 ± 0.35	0.80 ± 0.12	0.86 ± 0.02	0.66 ± 0.07	0.63 ± 0.05
		NS	NS	NS	NS	P < 0.02

The enzyme activity is expressed as μmoles of sodium pyruvate/mg protein/hr. Each value is the mean ± S. D. of 5 individual observations. NS denotes non-significance at 5% level.

Table 2

Alanine aminotransferase activity in snails in different concentrations of salinity

Animals	Control	Salinity Concentrations				
		0.001 M	0.002 M	0.003 M	0.004 M	0.005 M
Lymnaea	1.72 ± 0.30	1.56 ± 0.21	1.20 ± 0.86	4.16 ± 0.86	5.12 ± 0.79	4.76 ± 1.56
		NS	P < 0.02	P < 0.01	P < 0.01	P < 0.02
Planorbis	0.60 ± 0.15	0.54 ± 0.09	0.68 ± 0.07	0.64 ± 0.16	0.36 ± 0.09	0.32 ± 0.01
		NS	NS	NS	NS	P < 0.05
Viviparus	1.22 ± 0.11	1.86 ± 0.37	2.42 ± 0.50	1.66 ± 0.31	2.04 ± 0.09	1.96 ± 0.08
		P < 0.02	P < 0.02	P < 0.05	P < 0.01	P < 0.01

The enzyme activity is expressed as μmoles of sodium pyruvate/mg protein/hr. Each value is the mean ± S. D. of 5 individual observations. NS denotes non-significance at 5% level.

DISCUSSION

It is known that the tolerance of fresh water animals to different salinities varies with the species. The fresh water Crustacea, in general, tolerate wide ranges of salinity (Duval, 1925; Bogucki, 1934; Lockwood & Croghan, 1957; Ramamurthy, 1967). Among gastropods only *Pila globosa* is able to tolerate up to 37½% sea water (Ramamurthy, 1965). In contrast to the above findings, *Lymnaea* were unable to survive even at lower concentrations of salt water, indicating that they are mainly stenohaline. *Planorbis* and *Viviparus,* on the other hand, were able to survive in all concentrations, thereby showing their euryhaline nature. Mollusks, in general, have limited powers of anisosmotic extracellular regulation on account of the large permeable surfaces they have, through which salt and water exchange take place rapidly (Robertson, 1964; Ramamurthy, 1973). It was pointed out (Duchateau *et al.*, 1952) that free amino acids participate in the intracellular isosmotic regulation of mollusks. Subsequently, the involvement of free amino acids and their concentration in various salinities in other groups of invertebrates has been discussed in detail (Florkin, 1966; Florkin & Schoffeniels, 1965). The seasonal survival capacity of *Lymnaea* is probably due to fluctuations in the free amino acid composition in tissues of these animals.

It has been well documented that changes in the environmental salinity bring about significant changes in the activity and metabolism in poikilotherms (Florkin, 1960; Robertson, 1960; Lockwood, 1962; Kinne, 1964; Potts & Parry, 1964) resulting either in an increase or a decrease in the metabolic rate (Huggins & Munday, 1968). In *Lymnaea* the AAT activity was significantly decreased in 0.001 and 0.002 M salt water, indicating the sensitiveness of the enzyme at these concentrations. In other saline media the enzyme was unaffected. The AlAT activity showed significant decline at 0.002 M and increase in other saline media. This shows that more alanine than aspartic acid would have been made free in tissues to perform an osmotic role effectively at concentrations above 0.002 M. Consequently, the AlAT activity was enhanced. In *Planorbis,* both the enzyme activities behaved similarly in all saline media. The AAT activity was unaffected in all concentrations and so is the case with with the AlAT activity except at 0.005 M saline medium where it was significantly suppressed. *Planorbis* has a relatively greater ability to withstand the changes by carrying out anisosmotic extracellular regulation in salt concentrations of the medium. Hence the results indicate lesser involvement of free amino acids in the osmotic regulation in the salinity ranges studied. In *Viviparus* the 2 enzymes were different in their response to salinity, wherein the AAT activity was unaffected except at 0.005 M concentration and the AlAT activity was significantly increased in all concentrations of salt water. Since the concentration of free alanine in tissues of certain lamellibranchs increases several fold as salinity is increased (Allen, 1961; Lange, 1963; Lynch & Wood, 1966; Virkar & Webb, 1970), it is expected that in *Viviparus* also more alanine would have been accumulated, thus facilitating the enzyme to use all of the available substrate, resulting in pronounced enzyme activity. It seems more likely that alanine rather than aspartic acid is involved in the osmotic regulation in *Viviparus.*

It is evident from Tables 1 and 2 that the metabolic response of an animal to salinity varies with the species and also with the enzyme in question.

SUMMARY

1. Aspartate and alanine aminotransferase activities of tissue homogenate from 3 species of gastropods were determined colorimetrically in relation to salinity.

2. Both the enzyme activities showed varied responses to changes in salt water. Participation of alanine in the osmotic regulation is discussed. It is surmised that the metabolic response of an animal to the environmental salinity varies greatly with the species and also with the enzyme.

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